REMARKS

Claims 17-32 are pending.

Claim 17 is amended to (a) replace reference to a "pre-selected" with a "specific" chromosomal location; and, (b) to recite targeting to the specific chromosomal location is increased by at least two-fold over a PGK promoter-containing targeting vector having homology arms directing the PGK promoter-containing targeting vector to the same chromosomal location but having a drug resistance gene under control of a PGK promoter.

Claim 21 is amended to (a) replace reference to a "pre-selected" with a "specific" chromosomal location; (b) to recite that the homology arms direct the vector to location in a mouse ES cell; and, (c) to recite that specific chromosomal location directed by the homology arms is a chromosomal location where the targeting vector achieves at least a two-fold higher targeting than a PGK promoter-containing targeting vector having a drug resistance gene under control of a PGK promoter.

Claim 25 is amended to (a) replace reference to a "pre-selected" with a "specific" chromosomal location; and, (b) to recite that targeting frequency to the specific chromosomal location is increased at least two-fold higher than targeting frequency to the specific chromosomal location obtained using a method employing a PGK promoter-containing targeting vector having homology arms directing the PGK promoter-containing targeting vector to the specific chromosomal location but having a drug resistance gene under control of a PGK promoter.

Claim 29 is amended to (a) replace reference to a "pre-selected" with a "specific" chromosomal location; and (b) to recite that at least a two-fold higher number of mouse ES cells are correctly targeted than obtained by a method employing a PGK promoter-containing targeting vector having homology arms directing the PGK promoter-containing targeting vector to the same specific chromosomal location but having a drug resistance gene under control of a PGK promoter.

Support for the amendments to the claims can be found in the claims as filed for the amendment replacing "pre-selected" with "specific." Support for the amendments to claim 17 can be found at, for example, paragraph [0014], paragraphs [0042] through [0044] and Table 2 on page 11, in particular at columns 1, 2, and 8. Support for the amendments to claim 21 can be found at, for example, paragraph [0014], paragraph [0031], paragraphs [0042] through [0044] and Table 2 on page 11, in particular at columns 1, 2, and 8. Support for the amendments to claim 25 can be found at, for example, paragraph [0014], paragraphs [0042] through [0044] and

Table 2 on page 11, in particular at columns 1, 2, and 8. Support for the amendments to claim 29 can be found at, for example, paragraph [0014], paragraphs [0042] through [0044] and Table 2 on page 11, in particular at columns 1, 2, 4, and 5.

The amendments to the claims add no new matter, and the Examiner is respectfully requested to enter them.

Rejections Under 35 USC § 112, First Paragraph: New Matter

The Examiner rejected claims 17-32 as amended to include alleged new matter for reciting the phrase "pre-selected chromosomal location" instead of "specific chromosomal location." The Examiner considered that the term "specific" is not identical in scope to "pre-selected."

Applicants respectfully disagree with the Examiner that reciting that the chromosomal location for the targeting vector is "pre-selected" is not supported by the specification. Applicants note that the claimed targeting vectors contain homology arms, which are necessarily pre-selected to correspond with a specific chromosomal location. Nonetheless, solely to expedite prosecution, Applicants have amended the claim to recite "specific," as originally recited. Accordingly, the new matter rejection is moot.

Rejections Under 35 USC § 103(a)

The Examiner maintained his rejection of claims 17-32, asserting that the rejected claims are obvious in light of Rohozinski et al. (2002) Genesis, 32:1-7, in view of Tsirigotis et al. (2001) BioTechniques, 31:120-130 and Ghazizadeh et al. (1998) J. Invest. Dermat. 111:492-496, for the reasons stated in the Non-final Office Action dated 28 August 2006.

Applicants have amended the claims and submit that the amendments to the claims moot the Examiner's obviousness rejections. To the extent that the references cited and arguments made by the Examiner might be applied to the pending claims, Applicants address them below.

A *prima facie* case for obviousness requires that the differences between the rejected claim and the prior art are such that the subject matter ***as a whole*** would have been obvious at the time the invention was made. 35 USC § 103(a). Although a combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results, a claim composed of several elements is not proved obvious merely by demonstrating that each element is known in the art. KSR v. Teleflex, Inc., No. 04-1350, slip

op. at 14 (US, 30 April 2007). An explicit analysis should state whether there was an apparent reason to combine elements known in the art in the manner claimed. Id. However, "[e]lements may, of course, especially in chemistry ... take on some new quality or function from being brought into concert ..." A&P Tea Co. v. Supermarket Corp., 340 U.S. 147 (1950) (cited favorably in KSR v. Teleflex, Inc.), and "[a] combination of elements may result in an effect greater than the sum of the several effects taken separately," Anderson's Black Rock v. Pavement Co., 396 U.S. 57 (1969) (cited favorably in KSR v. Teleflex, Inc.). In an obviousness analysis, caution must be exercised to avoid *ex post* reasoning and distortion due to hindsight bias. KSR v. Teleflex, Inc., No. 04-1350, slip op. at 17.

The differences between the pending claims and the references cited (and assertions regarding the art) are substantial, such that the pending claims, as a whole, would not have been obvious to a person of ordinary skill at the time the invention was made.

The Examiner is referred to arguments made of record in Applicants' prior submissions, and to the declaration of David Frendewey dated 25 July 2006, which are incorporated herein by reference, except for Applicants' assertion that an "obvious to try" standard is impermissible, which is mitigated by the decision in KSR v. Teleflex, Inc., *supra*. But Applicants emphasize that there is no guidance in the art to suggest that targeting with a ubiquitin promoter-driven drug resistance gene will increase successful targeting at a specific chromosomal location in comparison with targeting ***the same specific chromosomal location*** with a PGK promoter-driven drug resistance gene. Accordingly, nothing in the art would motivate a person of ordinary skill to select a ubiquitin promoter among all available promoters in the art to enhance correct targeting at the specific chromosomal location where a PGK promoter-driven targeting construct provides lesser correct targeting.

Applicants describe using a ubiquitin promoter to drive expression of a drug resistance gene as part of a gene targeting construct that targets a specific chromosomal location (by virtue of selection of homology arms), which results in an increase in the number of ES cell colonies exhibiting drug resistance to a selection agent following introduction of the targeting vector (paragraph [0024]). The number of correctly targeted ES cell clones (*i.e.*, where the targeting construct is integrated at a specific chromosomal location, as opposed to a random integration) is a multiple of the number of drug resistant ES cell colonies and the frequency of targeting events [paragraph [0029]).

Applicants are the first to teach that changing the promoter driving or controlling expression of a drug resistance gene to a ubiquitin promoter (rather than a commonly used

promoter in a targeting vector, *e.g.,* a PGK promoter) significantly increases the ratio of targeted ES cell clones to total drug resistant ES cell clones (paragraph [0030]) when directed to the same chromosomal location as a commonly used art-recognized promoter (*e.g.*, a PGK promoter).

Applicants teach that the significant increase in the ratio of targeted ES cell clones to total drug resistant ES cell clones when a ubiquitin promoter is used instead of the PGK promoter (one of the most commonly employed promoters in the art), is an average of six-fold higher (paragraph [0041]), and spans from two-fold (Table 2, page 8, at col. 8, for gene designated "20" in col. 1) to 6.5-fold (Id., for gene designated "F" in col. 1).

Further, using a ubiquitin promoter rather than a PGK promoter results in a significant enhancement in targeting efficiency: the using a ubiquitin promoter results in an average of four-fold higher correct targeting to a specific locus (defined by the homology arms) as opposed to random insertion at any locus (arithmetic mean of numbers in Table 2, page 11, col. 8).

Thus, Applicants' invention not only increases the number of targeted ES cells over the prior art (*i.e.*, total of surviving ES cells expressing the drug selection marker, including those bearing both correctly targeted specific chromosomal locations and random insertion events at non-specific chromosomal locations; see last line of page 11, under Table 2), but also unexpectedly and significantly increase the number of targeted ES cells bearing the selection marker at the specific chromosomal location corresponding to the homology arms, in comparison to randomly-integrated constructs bearing the marker.

The features discussed above are nowhere disclosed in the references cited by the Examiner when considered either alone or in combination with one another or knowledge in the art, and the Examiner has not pointed to anything in the art that would suggest or motivate making the targeting construct as claimed.

The claims are now amended to recite that the method includes an enhancement of correct targeting to a specific chromosomal location of at least two-fold over correct targeting to the same specific chromosomal location seen in the art with a PGK promoter-driven drug selection gene, and to recite a targeting vector whose homology arms (directed to a specific chromosomal location) enhance targeting at least two-fold over a targeting vector with homology arms directing it to the same specific chromosomal location but having a drug resistance gene driven by a PGK promoter rather than a ubiquitin promoter.

Simply put, no references have been cited that disclose, teach, or suggest that a targeting vector with a ubiquitin promoter driving expression of a drug selection marker would

better promote correct targeting efficiency (more correctly targeted events at a specific chromosomal locus corresponding to the vector's homology arms as opposed to random insertions) than a targeting vector with homology arms directed to the same chromosomal locus but driven by the PGK promoter. Further, there is nothing in the art that would suggest this phenomenon and thereby motivate a person to select a ubiquitin promoter from all available promoters in the art. The number of available eukaryotic promoters is very large. See, for example, Perier et al. (1998) The Eukaryotic Promoter Database EPD, Nucleic Acids Res. 26/1:353-357, cited in the accompanying IDS and enclosed, which cites at least 173 promoters published in one journal alone as of 1986 (Perier at page 353, col. 1, first paragraph), and a total number of eukaryotic promoter entries in a eukaryotic promoter database of 1308, with vertebrate promoter entries numbered at 910 as of 1998 (see Perier at page 355, Table 1).

Moreover, as discussed previously, the fact that the ubiquitin promoter is active in many tissues has absolutely no bearing on whether it is silenced at a specific chromosomal location or not. The Examiner has not provided any reference or referred to any knowledge in the art that a ubiquitin promoter is more likely than any other promoter to drive better expression at a specific chromosomal location (regardless of the tissue) where a commonly employed eukaryotic promoter apparently does not effectively drive expression, or drives expression relatively poorly. The activity of the ubiquitin promoter in many tissues has absolutely no bearing on whether it will exhibit activity at a specific chromosomal location where a PGK promoter is silenced or repressed or otherwise inactive. Accordingly, the fact that the ubiquitin promoter works in many tissues cannot be a rational basis for selecting it to place in a specific chromosomal location where another commonly used (ubiquitously active, or constitutive) eukaryotic promoter is either not active or relatively weak. In fact, the inactive or weak promoter (*e.g.*, the PGK promoter) shares the very same feature (active in many tissues, constitutive) that the Examiner argues motivates the use of the ubiquitin promoter.

In light of the above, Applicants submit that the pending claims are not obvious in light of the references and arguments made by the Examiner with respect to the prior rejected claims.

Information Disclosure Statement

Applicants herewith submit an information disclosure statement and enclose WIPO and non-patent publications cited therein. To the extent that the references in the information disclosure statement might be applied to the pending claims, Applicants distinguish them below:

Johansen, T. et al. (1990) FEBS Lett. 267/2:289-294 ("Johansen 1990"). Johansen

1990 discloses eukaryotic expression vectors for expressing proteins in CHO cells and neuroendocrine CA77 cells. The ubiquitin promoter is used "[t]o achieve constitutive and ubiquitous expression of the inserted cDNA" (Johansen 1990 at page 291, col. 2, first paragraph), and a drug resistance gene is included on the expression vector (such as ampicillin resistance or neomycin resistance, see Figs 1 and 2), though not driven by the ubiquitin promoter (see, for example, Fig. 2). None of the vectors are targeting vectors, and no mouse ES cells are transfected.

International Patent Application Publication No. WO 98/32869 A1 by Johansen (the "Johansen Application"). The Johansen Application discloses using a ubiquitin promoter to stably and efficiently express genes for therapeutically active proteins in the CNS (see Johansen Application at page 3, lines 13-21), and cells transfected with ubiquitin-driven retroviral expression vectors (Johansen Application at page 3, lines 22-32). Expression vectors having drug selection markers are described, but when described they are not operably linked to a ubiquitin promoter (see Johansen Application at page 8, lines 16-22). The Johansen Application does not disclose using ubiquitin in a targeting vector having homology arms to direct it to a specific chromosomal location, but instead in connection with randomly-integrating viral constructs. Further, mouse ES cells are not mentioned. There is no disclosure regarding any ability of a ubiquitin promoter to provide enhanced targeting at a specific chromosomal location over targeting using a marker driven by a PGK promoter.

Lois, C. et al. (2002) Science 295:868-872 ("Lois"). Lois discloses lentiviral vectors for infecting single-cell mouse embryos with green fluorescent protein (GFP) driven by a ubiquitin C promoter, selected because it "was found to provide the most reliable expression across different cell types" (Lois at page 869, col. 1, first full paragraph). Lois does not disclose targeting constructs, targeting in ES cells, or enhancement in targeting at a specific chromosomal locus over, for example, a PGK promoter.

Spenger, A. et al. (2004) Protein Expression & Purification 38:17-23 ("Spenger"). Spenger illustrates that, even after the filing date of the instant application, the art recognized that ubiquitin promoters would not always necessarily provide the best activity. For example, Spenger discloses that expression of a CMV, an RSV, and an SV40 promoter each was better in COS cells than the ubiquitin C promoter in an expression construct (see Spenger at page 20, Table 2, and col. 1, second full paragraph).

Yew, N. et al. (2001) Molecular Therapy 4/1:75-82 ("Yew 2001"). Yew 2001 discloses using a ubiquitin B promoter in a plasmid DNA vector for transfecting lung cells, wherein the

ubiquitin promoter drives expression of the marker gene human SEAP (secreted alkaline phosphatase), provides lower but more stable transgene expression over time in the lung cells (see Yew 2001 at page 77, col. 1, first paragraph). Modifying the ubiquitin B promoter with a CMV enhancer (a hybrid promoter) resulted in undiminished robust expression for at least 12 weeks (Yew 2001 at page 77, col. 2, middle paragraph). Transducing liver cells with a reporter driven by the hybrid promoter in SCID mice resulted in similar findings (Yew 2001 at page 78, first paragraph). Thus, Yew 2001 discloses hybrid ubiquitin promoters for eukaryotic expression constructs, but not in targeting vectors and not in mouse ES cells, an effect presumably due to addition of CMV enhancer elements that comprise binding sites for certain trans factors (Yew 2001 at page 79, col. 2, last paragraph), since omission of the CMV enhancer elements reduces the effectiveness of the ubiquitin promoter.

US Patent Application Publication No. 2004/0047846 by Hyde ("Hyde"). Hyde discloses vectors having a human ubiquitin C promoter operably linked to a gene for a therapeutic agent for use in airway disease therapy (see Hyde at paragraph [0009]). Hyde discloses plasmid expression vectors and viral vectors, including "integrating gene transfer vector[s]" (see Hyde at paragraph [0014]), and a vector having a ubiquitin promoter driving a drug resistance gene (see Hyde at paragraph [[0097]), but not a targeting vector for a specific chromosomal location in a mouse ES cell (see Hyde at paragraph [0011]-[0015]). There is no disclosure of a ubiquitin promoter driving a drug resistance gene in a targeting vector, wherein targeting efficiency at a specific chromosomal location is enhanced over a corresponding targeting vector with drug resistance driven by a PGK promoter.

US Patent No. 6,667,174 to Yew (the "Yew Patent"). The Yew Patent discloses hybrid promoters that have a ubiquitin promoter in connection with enhancers from other promoters, *e.g.*, the human and mouse CMV promoters, which display persistent expression at significantly higher levels than driven by the ubiquitin promoter alone (see, *e.g.*, the Yew Patent at col. 3, lines 17-35). Many uses are mentioned (see, *e.g.*, the Yew Patent at col. 5, line 53 to col. 6, line 6), but targeting vectors for placing a ubiquitin promoter-driven drug selection gene at a specific chromosomal location are not mentioned. The Yew Patent does not disclose a targeting vector for mouse ES cells having a ubiquitin promoter driving expression of a drug resistance gene and homology arms for a specific chromosomal location.

US Patent No. 6,063,598 to Enenkel et al. ("Enenkel"). Enenkel discloses hamster ubiquitin S-27A promoters with or without regulatory sequences for making proteins in hamster cells (see Enenkel at col. 1, line 62 to col. 2, line 14). The promoters are to be used in

expression constructs in host cells (see Enenkel at col. 2, line 60 to col. 3, line 10), but mouse ES cells are not mentioned. Using the promoter in many vectors is mentioned, but not in a vector for targeting (*i.e.*, having homology arms) to a specific chromosomal location in an ES cell (see, *e.g.*, Enenkel at col. 5, line 27 to col. 6, line 12).

Perier et al. (1998) Nucleic Acids Research 26/1:353-357 ("Perier"). Perier is provided to illustrate that as of 1998 there were at least 1308 promoter entries in a eukaryotic promoter database (Perier at Table 1), at least 910 of which were in connection with vertebrate promoters (Table 1, item 6).

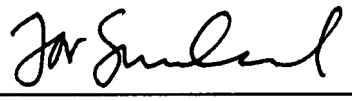
Conclusion

It is believed that this document is fully responsive to the Office action dated 05 June 2007. It is believed that the claims are now in condition for allowance, and such action is respectfully urged.

Fees

Applicants submit that no fee other than the RCE fee and one-month extension fee are due. If any further fees are due, or overpayment has been made, please charge, or credit, Deposit Account No. 18-0650 in the amount of the overpayment or fee deficiency.

Respectfully submitted,



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